PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JW01060WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/003232	International filing date *(day/month/year)* 26.07.2004	Priority date *(day/month/year)* 24.07.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant MEDICAL BIOSYSTEMS LTD. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.03.2005	28.07.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Helliot, B Telephone No. +49 89 2399-7793

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003232

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-14 as originally filed

Sequence listings part of the description, Pages

1 as originally filed

Claims, Numbers

1-13 as originally filed

Drawings, Sheets

1/2, 2/2 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003232

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

ITEM V:

1. INTRODUCTION

The following documents (D1-D2) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: WO-A-00/70073
D2: WO-A-00/53805

The present application relates to methods for determining the sequence of a polynucleotide or detecting variations between polynucleotide sequences.

2. NOVELTY and INVENTIVENESS (Art. 33(2) and (3) PCT)

a. No cited prior-art document discloses a method for identifying the sequence of a target polynucleotide comprising measuring the time taken by the polymerase to bind and subsequently to dissociate from the target polynucleotide in presence of one of the four nucleotides.
Therefore, the subject-matter of independent **claim 1,** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of **claim 1** does involve an inventive step over the prior art (Art. 33(3) PCT).

D1, which is considered as the closest prior art, relates to a method for sequencing a target nucleic acid molecule having a plurality of nucleotide bases wherein:
i) the method comprises (a) providing a complex of a nucleic acid polymerizing enzyme and the target nucleic acid molecule oriented with respect to each other in a position suitable to add a nucleotide analog at an active site complementary to the target nucleic acid; (b) providing a plurality of types of nucleotide analogs proximate to the active site, wherein each type of nucleotide analog is complementary to a different nucleotide in the target nucleic acid sequence; (c)

polymerizing a nucleotide analog at an active site, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid, leaving the added nucleotide analog ready for subsequent addition of nucleotide analogs; (d) identifying the nucleotide analog added at the active site as a result of said polymerizing; and (e) repeating said providing a plurality of types of nucleotide analogs, said polymerizing, and said identifying so that the sequence of the target nucleic acid is determined (claim 1);
ii) in accordance with the present invention, it is possible to distinguish the event of binding of a nucleotide and its incorporation into nucleic acid from events just involving the binding (and subsequent rejection) of a mismatched nucleotide, because the rate constants of these two events are drastically different because an event of a mismatched binding of a nucleotide analog will be much shorter in time than the event of incorporation of the correct base (p. 22, l. 20-26).

The subject-matter of **claim 1** differs from that of D1 in that:
- the method uses only one nucleotide; and
- the time taken by the polymerase to bind and subsequently to dissociate from the target polynucleotide is monitored.

Thus, the technical problem to be solved by the subject-matter of said **claim 1** may be regarded as providing an alternative method to that of D1.

In view of the absence of any indication in the prior art suggesting the monitoring of polymerase-target interaction in presence of only one nucleotide, it is not obvious for the skilled person to develop a method for identifying the sequence of a target polynucleotide comprising measuring the time taken by the polymerase to bind and subsequently to dissociate from the target polynucleotide in presence of one of the four nucleotides as disclosed in the method of **claim 1**.
Therefore, the subject-matter of **claim 1** involves an inventive step (Art. 33(3) PCT).

b. Dependent **claims 2-13** further define specific embodiments of the novel and inventive method of claim 1. Dependent **claims 2-13** are hence also considered to meet the requirements of Art. 33(2) and (3) PCT.